

Cinnamaldehyde Enhances in vitro parameters and augments in vivo protection against avian Coccidiosis

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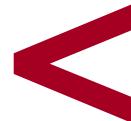
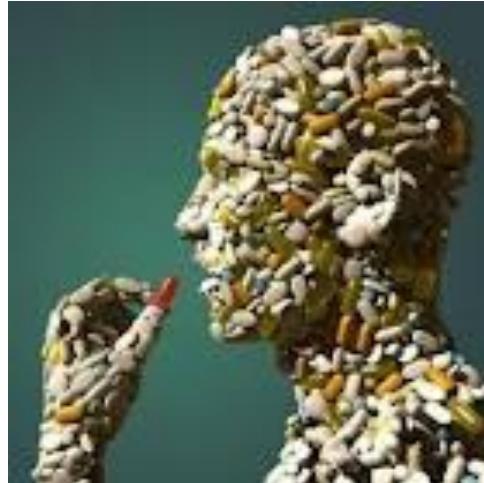
INTRODUCTION

Coccidiosis:

**economically important disease of poultry industry
annual economic loss > \$ 3 billion world wide.**



**Increasing governmental regulation of drug use
> Alternative ways to control animal diseases are needed.**



Dietary supplementation such as safflower leaf, plum, anethol, mushroom, capsaicin, curcumin, etc enhance immunity in chickens (Lee et al., 2008,2010a,b,2011a,b).

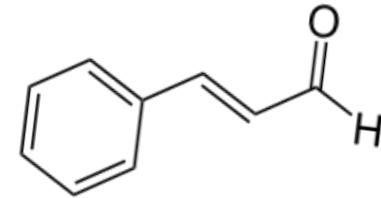


Cinnamon treated gastritis and inflammatory diseases.

Cinnamaldehyde (CINN): major constituent of cinnamon, strong antibacterial activity, improved ileal digestibility and gastrointestinal mucosa health.

Hypothesis:

Dietary feeding with CINN may provide an alternative to drug strategy to mitigate intestinal damage caused by avian coccidiosis in poultry.



OBJECTIVES

- **To investigate immune enhancing potential of CINN on avian innate immunity and to develop novel immunomodulation strategies to enhance intestinal health in young broiler chickens.**

- **To investigate the effect of dietary feeding of CINN of young broiler chickens in avian coccidiosis disease challenge model.**

< In vitro >

Material

Purified Cinnamaldehyde (CINN) from Pancosma was used after dialysis against PBS for 48 hours.

CINN sample was filtered through a 0.45 µm filter and serially diluted in PBS for in vitro evaluation.

Methods

Splenocyte proliferation and tumor cell growth inhibition were assessed using WST-8 (Cell-Counting Kit-8®).

Nitric oxide production by macrophages was measured using Griess reagent.

Sporozoite viability was measured using trypan blue dye exclusion test.

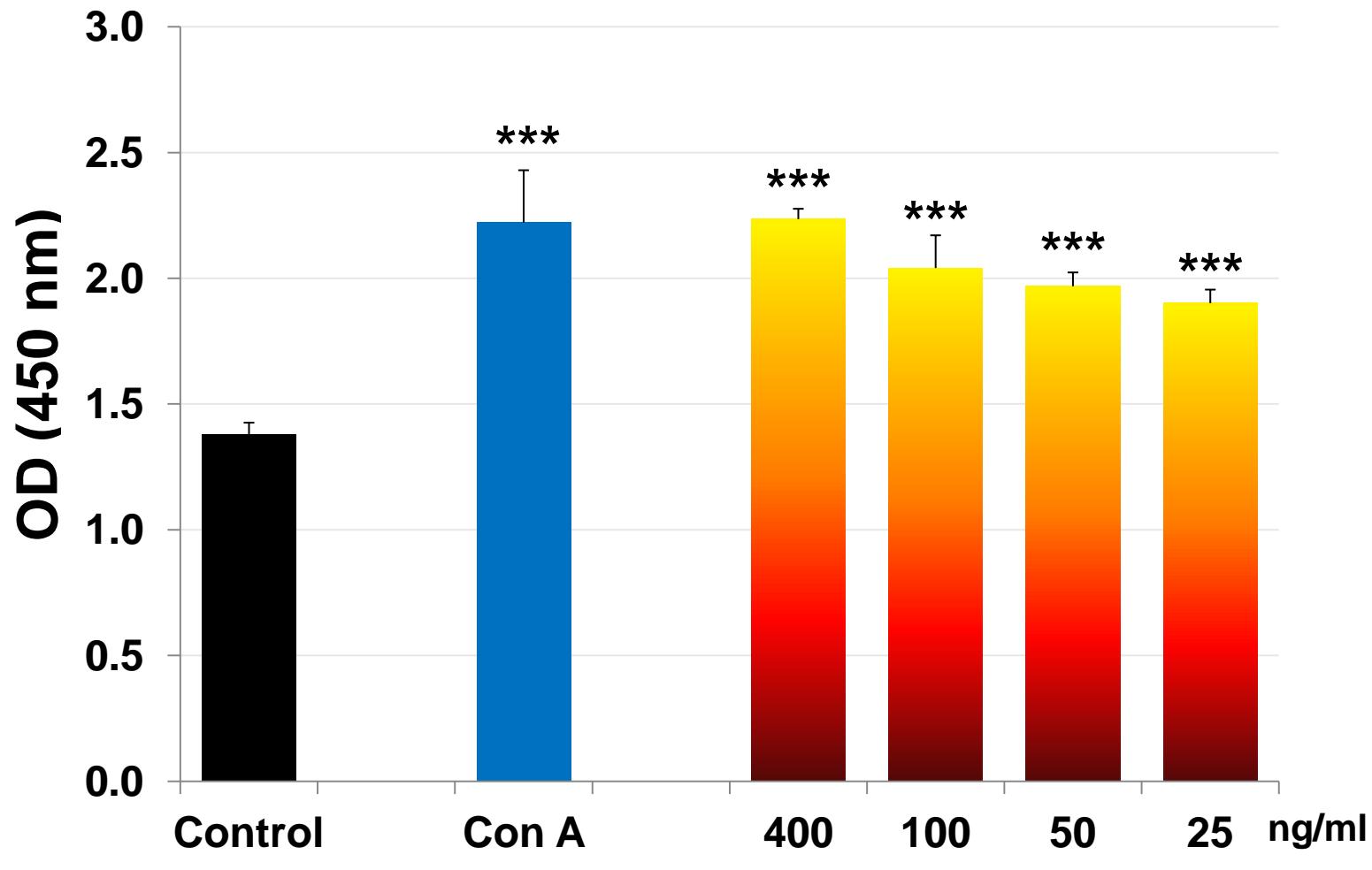


Fig. 1. Effects of CINN on splenocyte proliferation (2.5×10^6 /well, 48 hrs). *** $P < 0.001$

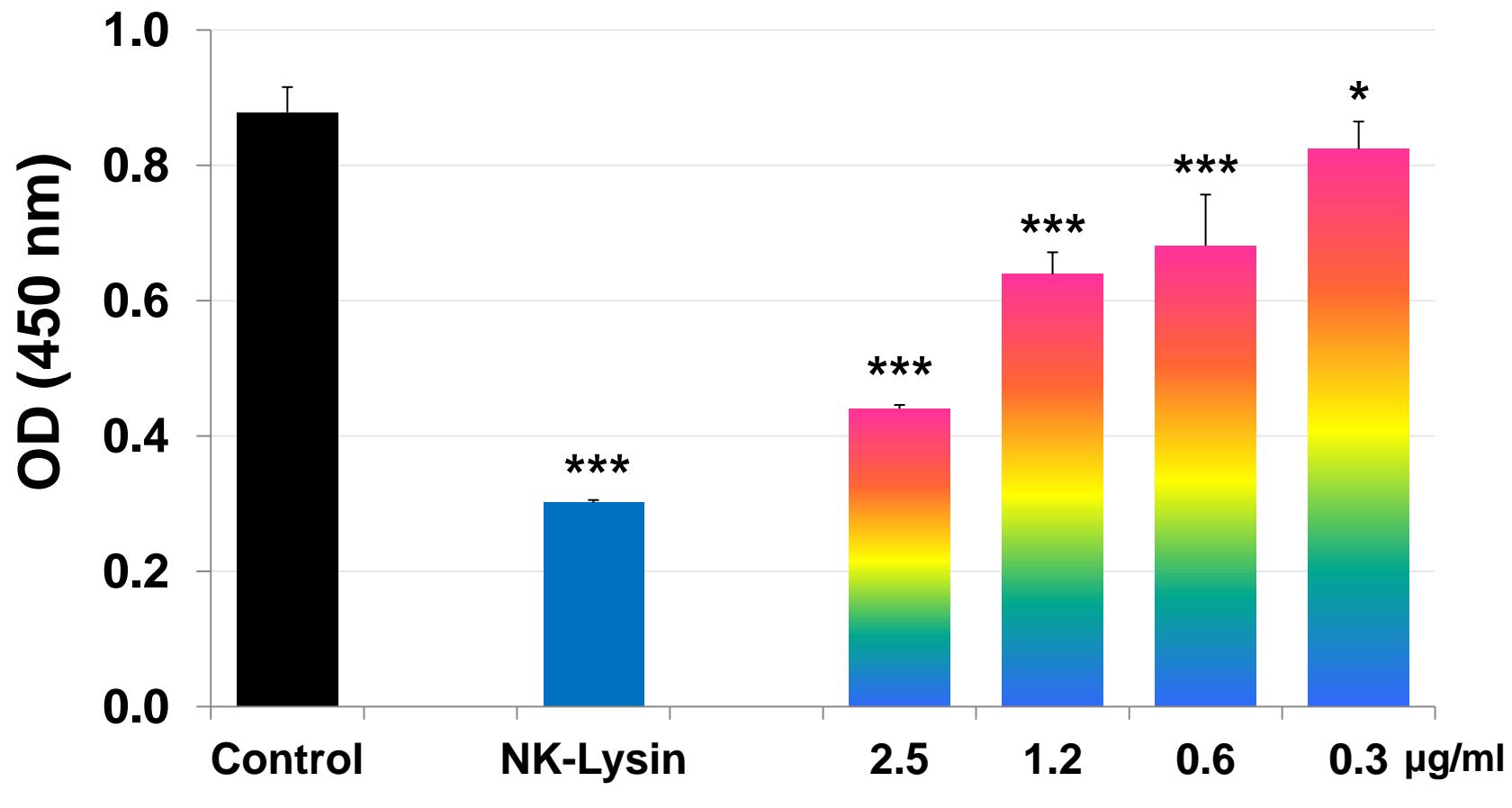


Fig 2. Effects of CINN on tumor cell growth ($1 \times 10^5/\text{well}$, 48 hrs).
*** $P < 0.001$, * $P < 0.05$

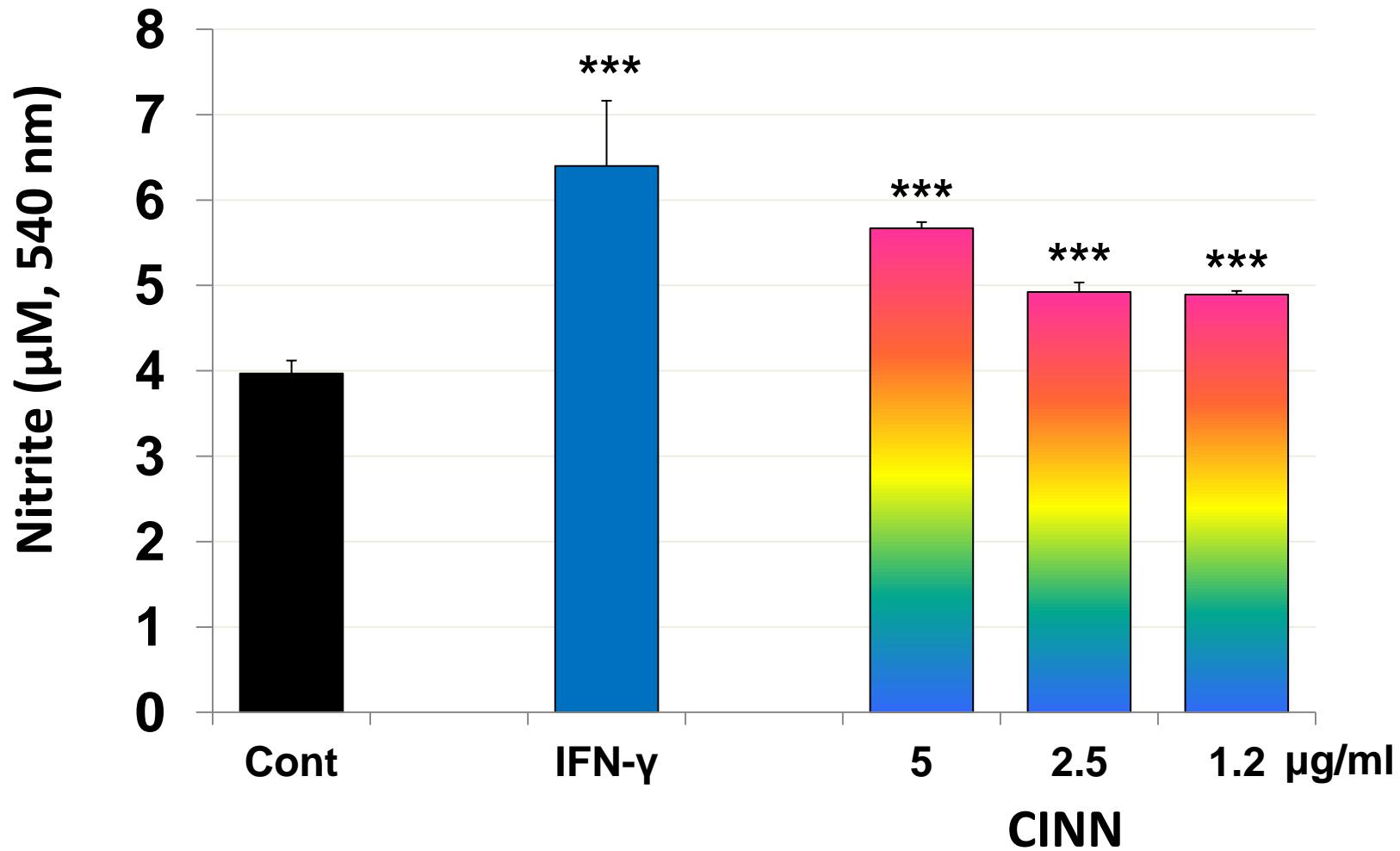


Fig 3. Effects of CINN on NO production ($1 \times 10^5/\text{well}$, 24hrs).

*** $P < 0.001$

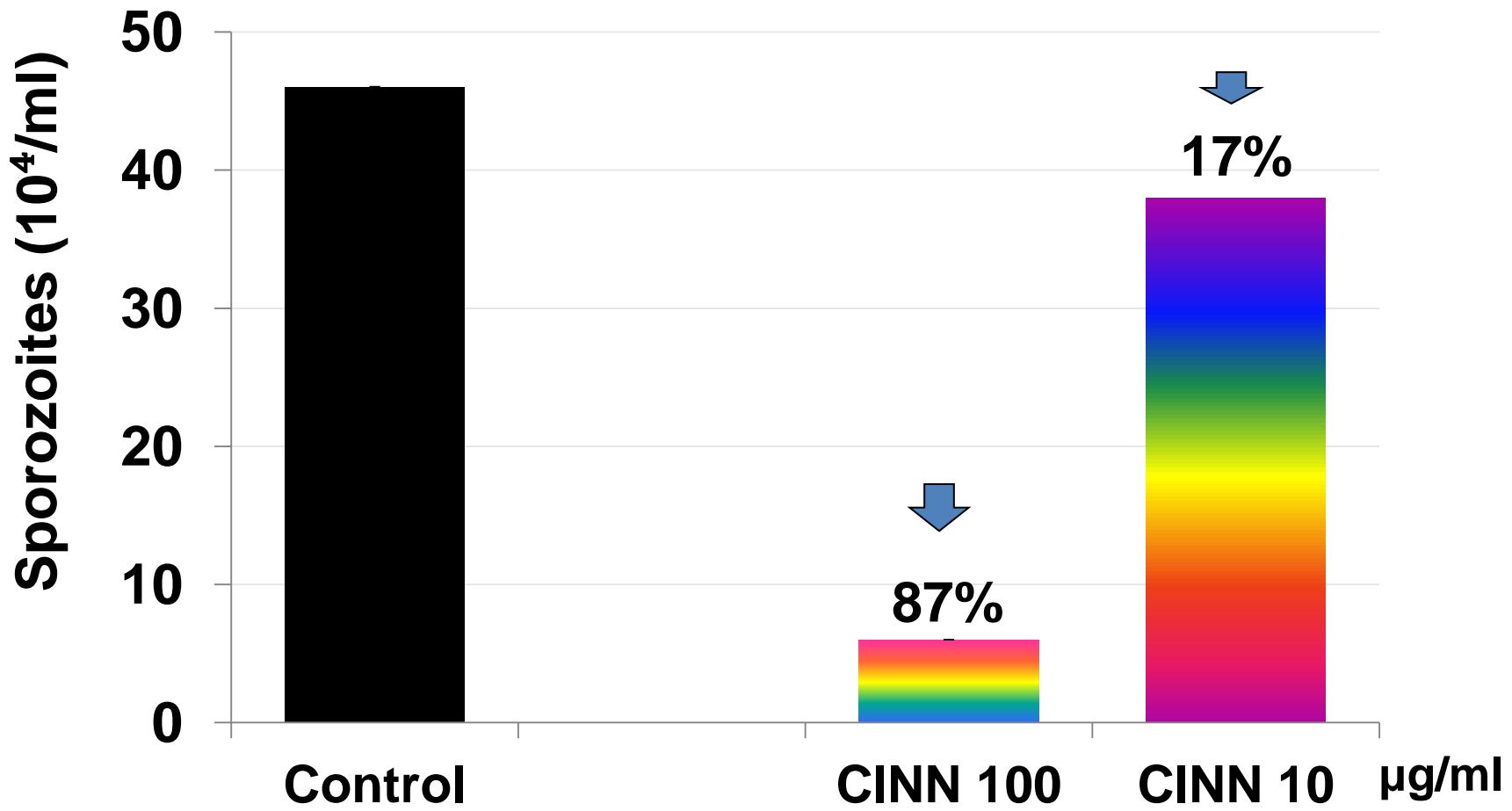


Fig. 4. Anti-parasitic properties of CINN. Numbers on the bars indicate the percentage inhibition against the media control.

< In vivo >



Ross
(n=20/group)

Body Weight

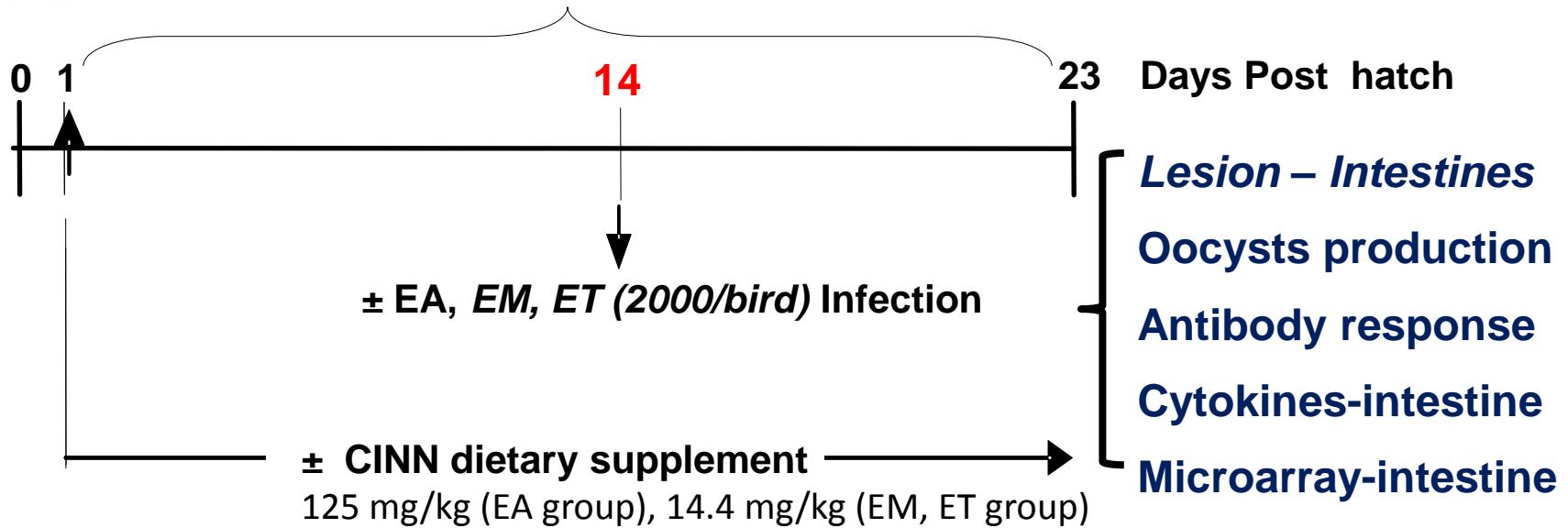


Fig 1. Schematic outline of the experimental design.

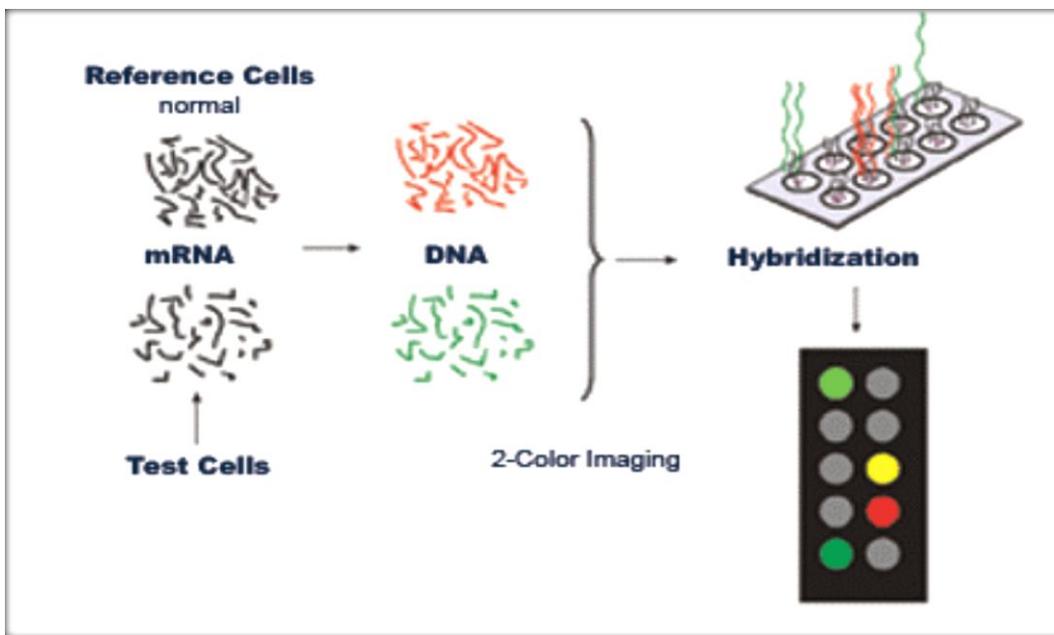
Fig 2. Analyzed Factors

**Body weight gain
between
0 & 9 days
postinfection
(dpi)**

**Fecal oocysts
at 6-9 dpi using
McMaster
chamber &
Lesion score
at 9 dpi**

**Serum Ab
to *Eimeria*
protein
at 9 dpi
using ELISA**

**Cytokine and
gene
expression
in intestine
at day 0
using RT-PCR**



**Bioinformatic
Analysis**
using GeneSpring
GX 7.3 &
informatics tools

Table 1. Oligonucleotide primers used for qRT-PCR of chicken cytokines.

RNA target	Primer sequences	PCR product size (bp)	Accession no.
GAPDH			
Forward	5'-GGTGGTGCTAAGCGTGTAT-3'	264	K01458
Reverse	5'-ACCTCTGTCATCTCTCCACA-3'		
IL-1 β			
Forward	5'-TGGGCATCAAGGGCTACA-3'	244	Y15006
Reverse	5'-TCGGGTTGGTTGGTGATG-3'		
IL-6			
Forward	5'-CAAGGTGACGGAGGAGGAC-3'	254	AJ309540
Reverse	5'-TGGCGAGGAGGGATTCT-3'		
IL-15			
Forward	5'-TCTGTTCTTCTGTTCTGAGTGATG-3'	243	AF139097
Reverse	5'-AGTGATTGCTTCTGTCTTGGTA-3'		
IFN- γ			
Forward	5'-AGCTGACGGTGGACCTATTATT-3'	259	Y07922
Reverse	5'-GGCTTGCCTGGATTC-3'		

Table 2. The composition of 10K AVIELA microarray V2 and array images scanned with Cy3 and Cy5 channels

Type of cDNA clones	Number of clones	Number of spots	Detail description
IEL ESTs	9,668	19,336	6,654 genes and 3,014 singleton ESTs
Controls	6	76	GAPDH, β-actin, soybean genes, and vectors
Blanks	N/A	494	Spotting solution only
Total elements	9,674	19,906	

+ 474 spot elements from LPS-stimulated macrophages and direct PCR clones of immune-related genes (*Appl Microbiol Biotechnol* 2003;62:392-399)

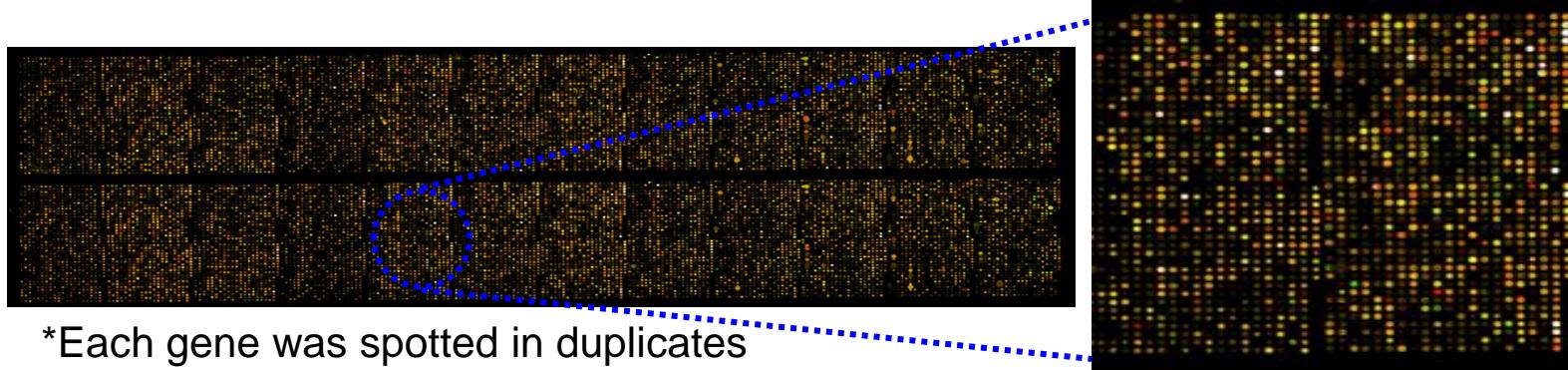
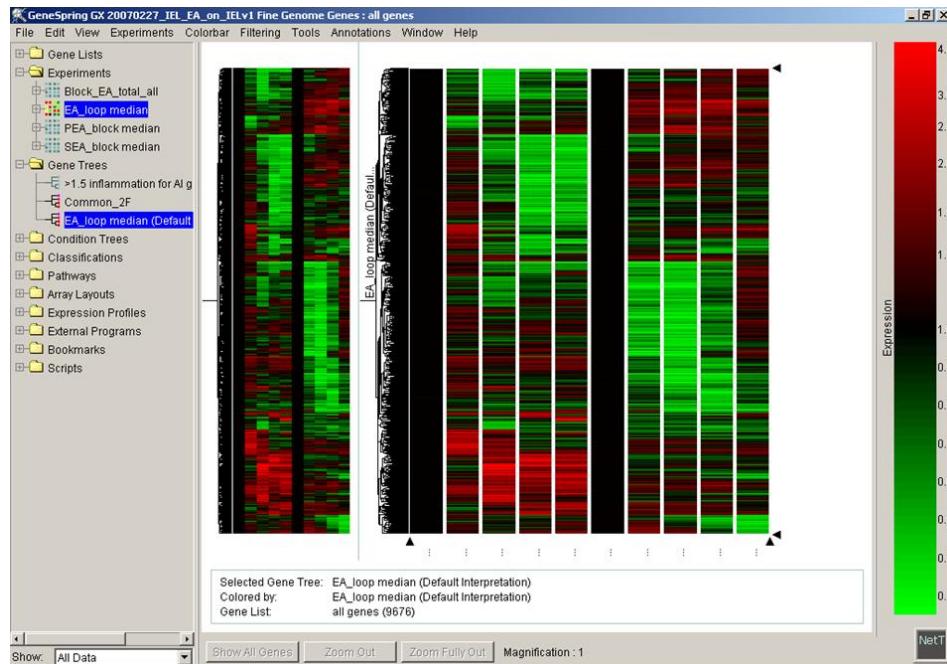
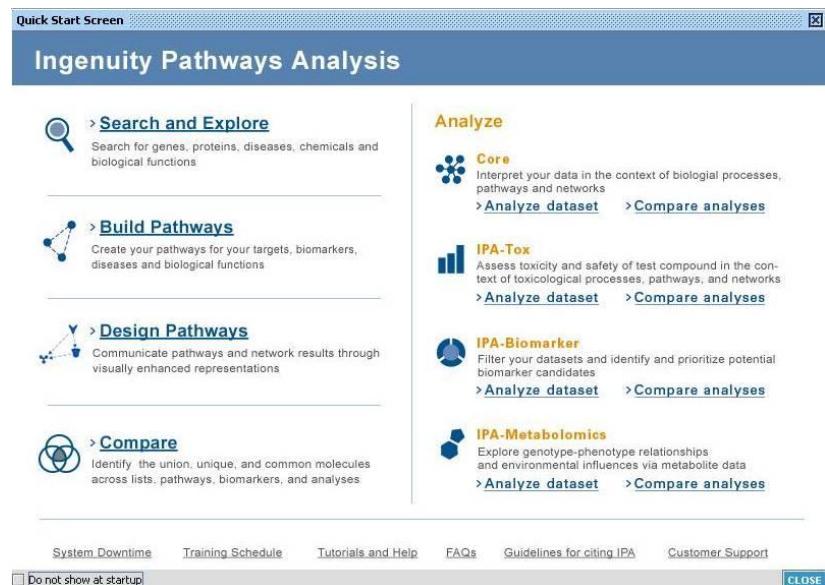


Fig 3. Data normalization and analysis

Statistical and bioinformatic analysis using GeneSpring GX 7.3 and informatics tools



Metabolomics analysis using Ingenuity Pathways Analysis



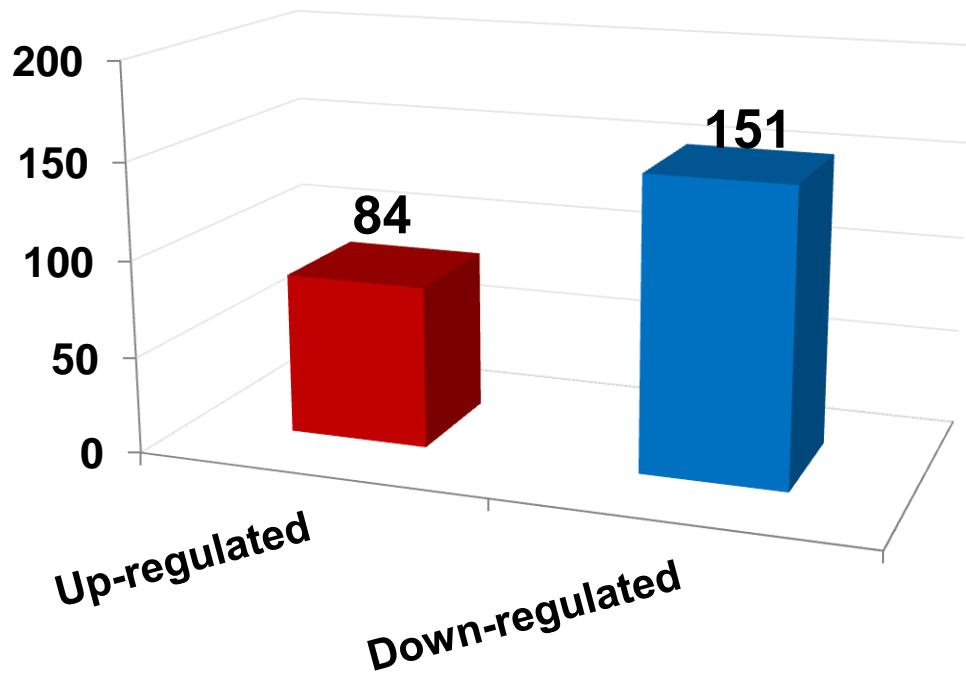


Fig 4. Number of transcripts affected by CINN feeding

Fig 5. Gene ontology analysis for the genes >2 fold up- or down-regulated by CINN

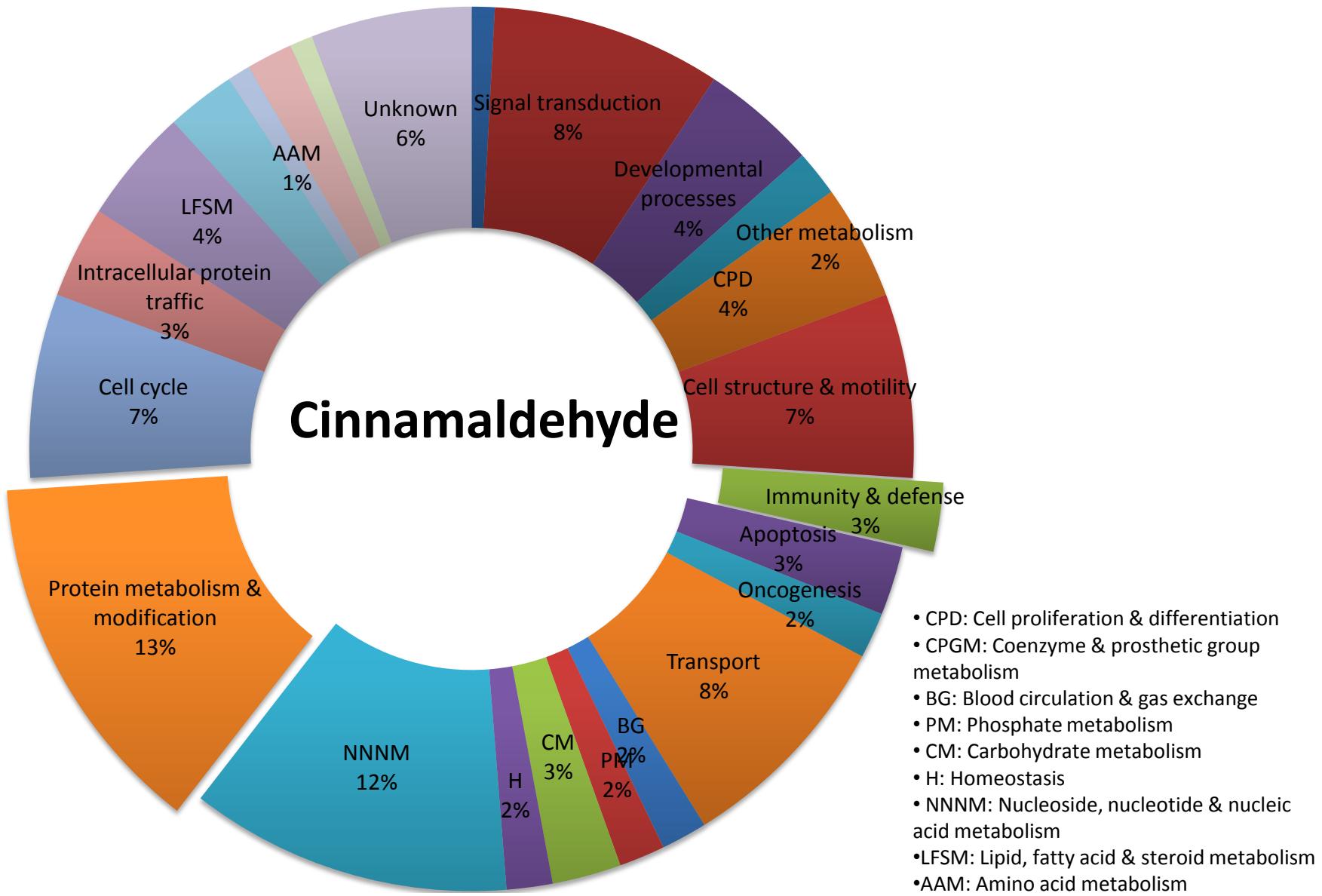


Table 3. Network analysis to assess the CINN effects

ID	Molecules in Network	Score	Focus Molecule	Top Functions
1	↑ADAM9, ↑AGRN, ↓carbon dioxide, ↓CBX3, ↓CCT2, ↓CD2, CD48, CD163, Cl2, ↓CLTB, ↑CSNK2A2, ↓DPP4*, dystroglycan, ERK1/2, F11R, ↓GNAS, HIST3H3 (includes EG:8290), Histone h3, ↑HSP90B1, Insulin, ↓LAMC1, Laminin, LARGE, MIR124, ↓PAFAH1B1, PDCL, PPP1R8, ↓PRSS2 (includes EG:5645), PSMA3, ↑SFRP1, ↑SH3KBP1, ↑SURF4, Top2, Tubulin, ↓UHRF1	39	18	Antigen Presentation, Humoral Immune Response, Inflammatory Disease
2	A1CF, ↓ARHGAP19, ↓ASB3, ATP5B, ATXN3, ↓C20RF49, Ca2+, ↓CUGBP2, FTH1, ↓FUNDC2 (includes EG:65991), ↓GATAD2A, HLTf, HNF4A, ↓HNRNPR, ↑IL22RA1, JUN, K+, KAT2B, KCNN2, MPP1, NR4A1, NSF, ↓P2RX4, P2RX6, progesterone, PSMA3, PSMC4, SGK1, ↓SLC24A2, ↓SNAPC5, ↓STK25, SYT2, TGFB1, ↓TMOD3, ↓VAPB	28	14	Cardiovascular System Development and Function, Tissue Morphology, Drug Metabolism
3	↓2-oxoglutaric acid, ADRM1, beta-estradiol, ↓BUB3, ↓DDX1, EGFR, ELF3, ↓FADD, ↓FUT8, ↑FXYD2, HIF1A, hydrogen peroxide, IL6, NR3C1, ↓OGDH, PSMA4, PSMA5, ↓PSMA6, PSMA7, PSMB1, PSMB2, PSMB4, PSMB6, PSMB7, PSMC4, PSMD6, RB1, ↑SFRS7, ↑SIK1, SLC2A4, ↓SLC34A2, ↑STK4, ↓UBE2D1, UCHL5, ↓USP4	27	14	Cell Death, Gene Expression, Cellular Development
4	amino acids, ↓CALM1, CD163, CLTC, ↑CTDP1, DAPK2 (includes EG:23604), ↓EPN2, Ferritin, GH1, HBA1, ↑HBB (includes EG:3043), HBB-AR, HBG1, HBG2, HBQ1 (includes EG:3049), ↓HBZ, HP, ↓IGH-VS107, IL1A, INS1, LNPEP, ↑MAGMAS, NME3, ↓NUBP2, ↓PDP2, PPEF2, PTP4A3, ↓RAB3GAP2, retinoic acid, ↓SGTA, ↑SMYD5, SPP1, SRGN, STK38, ↓UGT8	26	13	Cell Morphology, Connective Tissue Development and Function, Cellular Function and Maintenance
5	↓HERC4, HERC6	2	1	
6	↓COQ5, methyltransferase	2	1	
7	3-hydroxy-3-methylglutaryl-coenzyme A, coenzyme A, hydroxymethylglutaryl-CoA hydrolase, ↑meglitol, succinate-hydroxymethylglutarate CoA-transferase, succinic acid, succinyl-coenzyme A, water	1	1	Drug Metabolism, Small Molecule Biochemistry, Lipid Metabolism

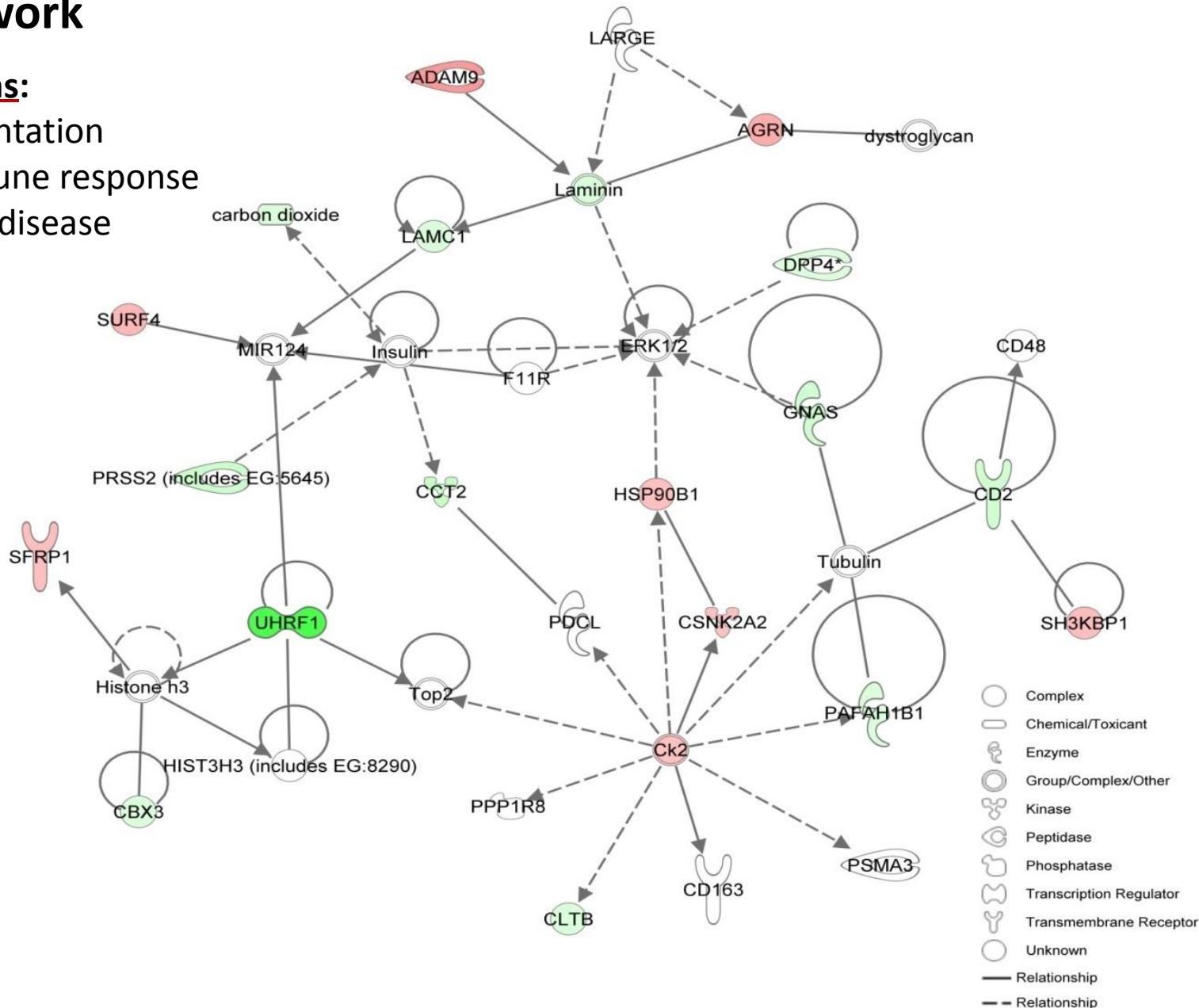
Fig 6. Network

* Top functions:

Antigen presentation

Humoral Immune response

Inflammatory disease



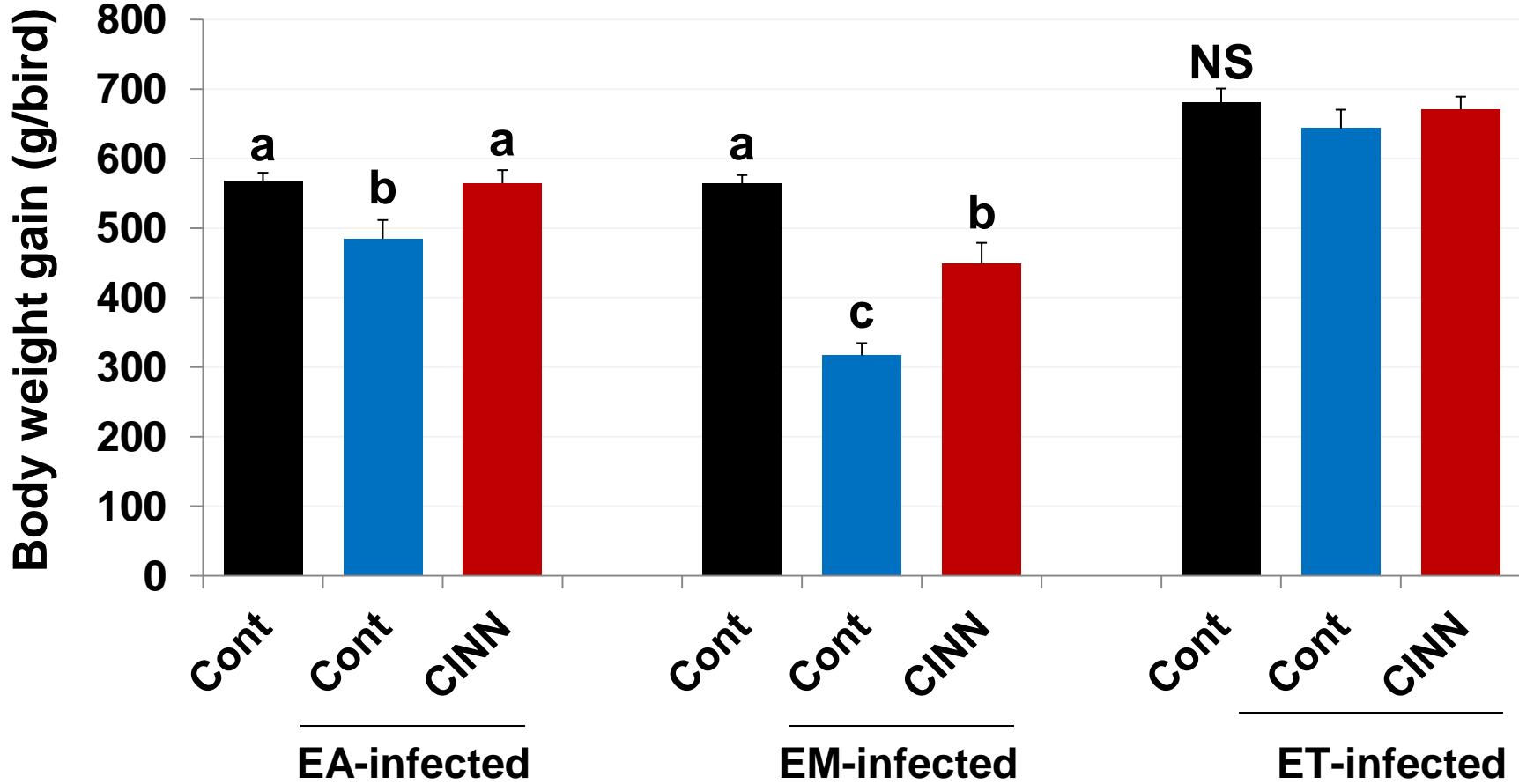


Fig 7. Effect of CINN on weight gain in EA and EM-infected chickens ($P < 0.05$)

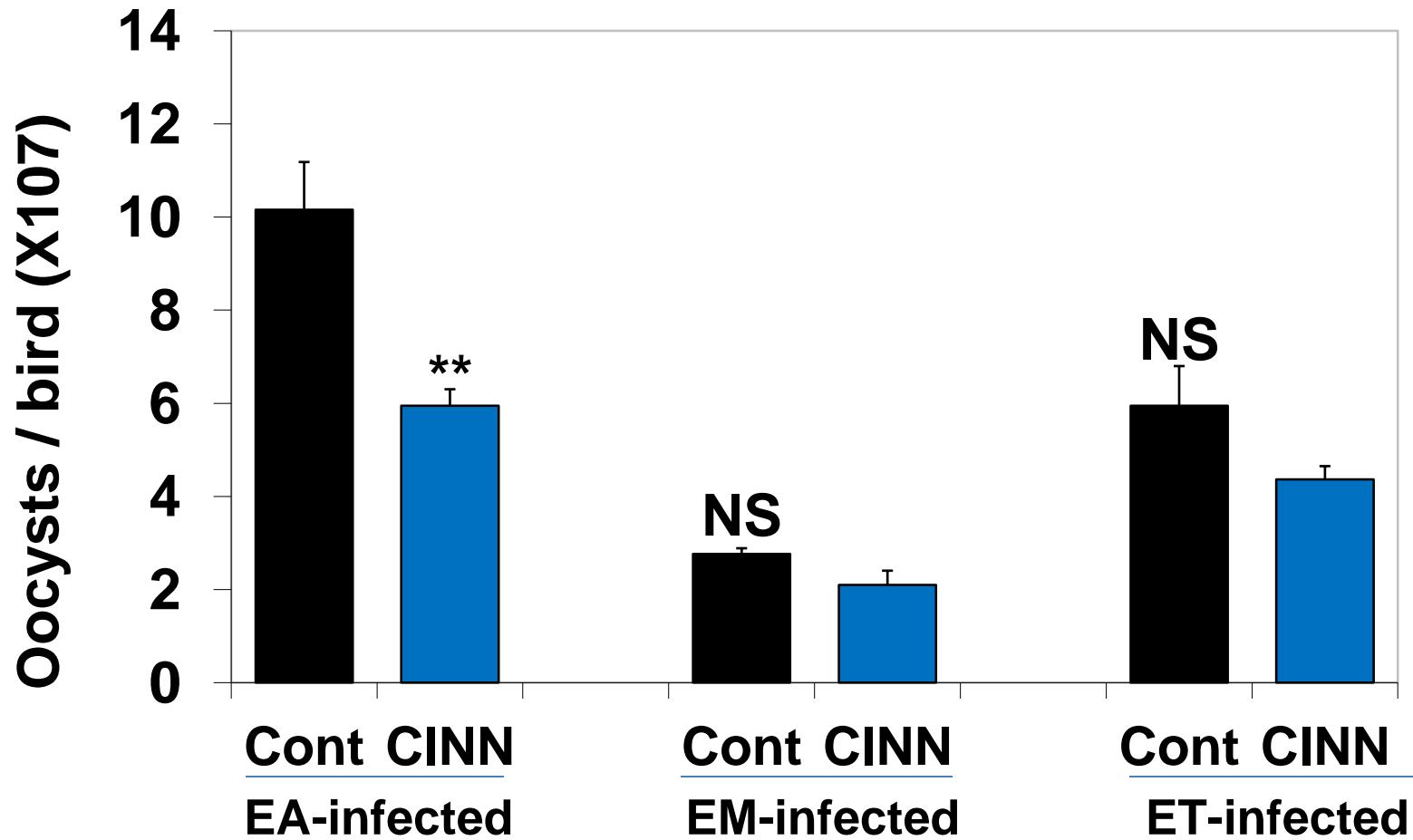


Fig 8. CINN feeding reduced oocyst shedding in EA-infected chickens. ** $P < 0.01$.

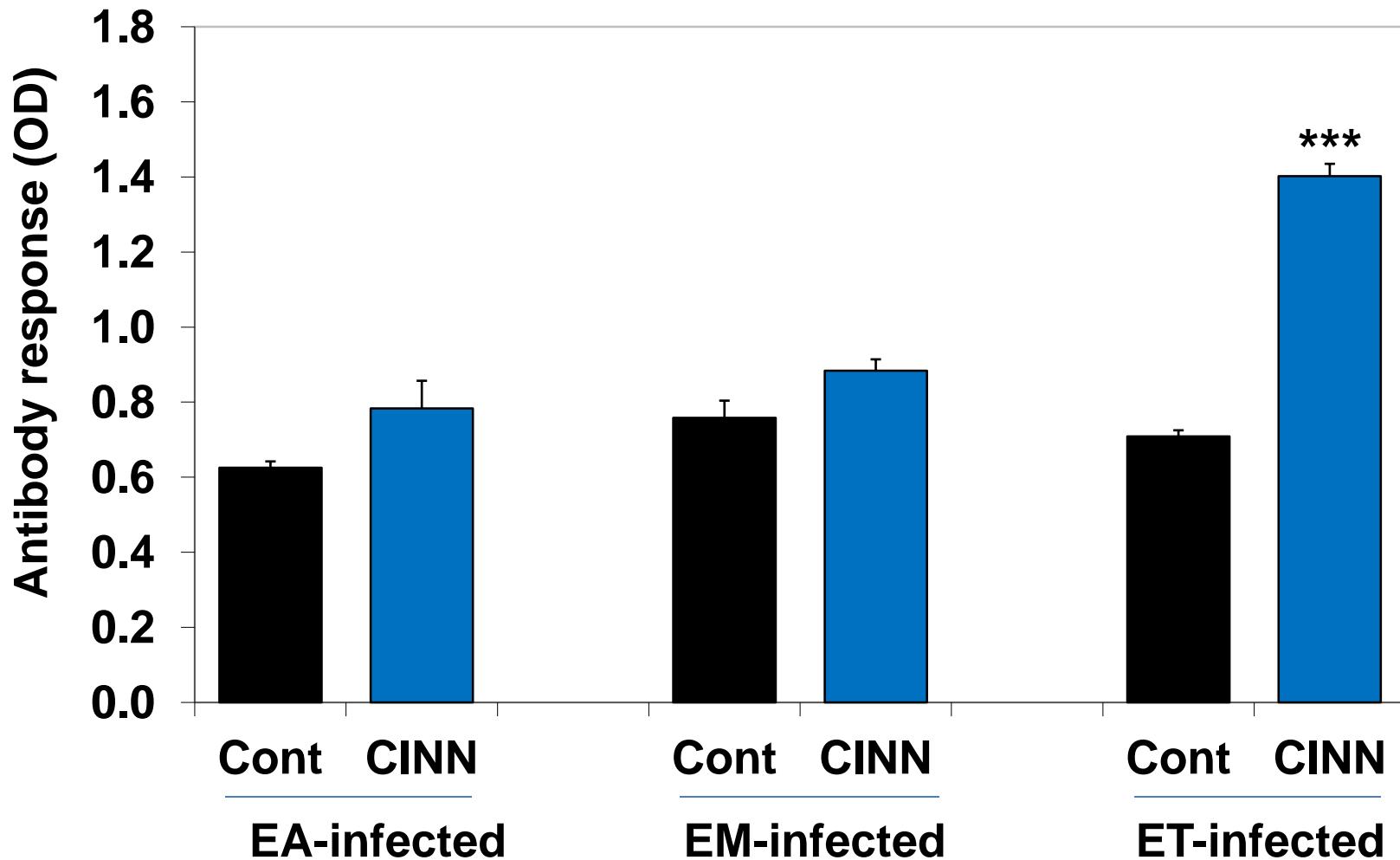


Fig 9. Effect of dietary CINN on serum antibody (EtMIC2) responses following infection with *EA*, *EM*, and *ET*. * $P < 0.05$, * $P < 0.001$.**

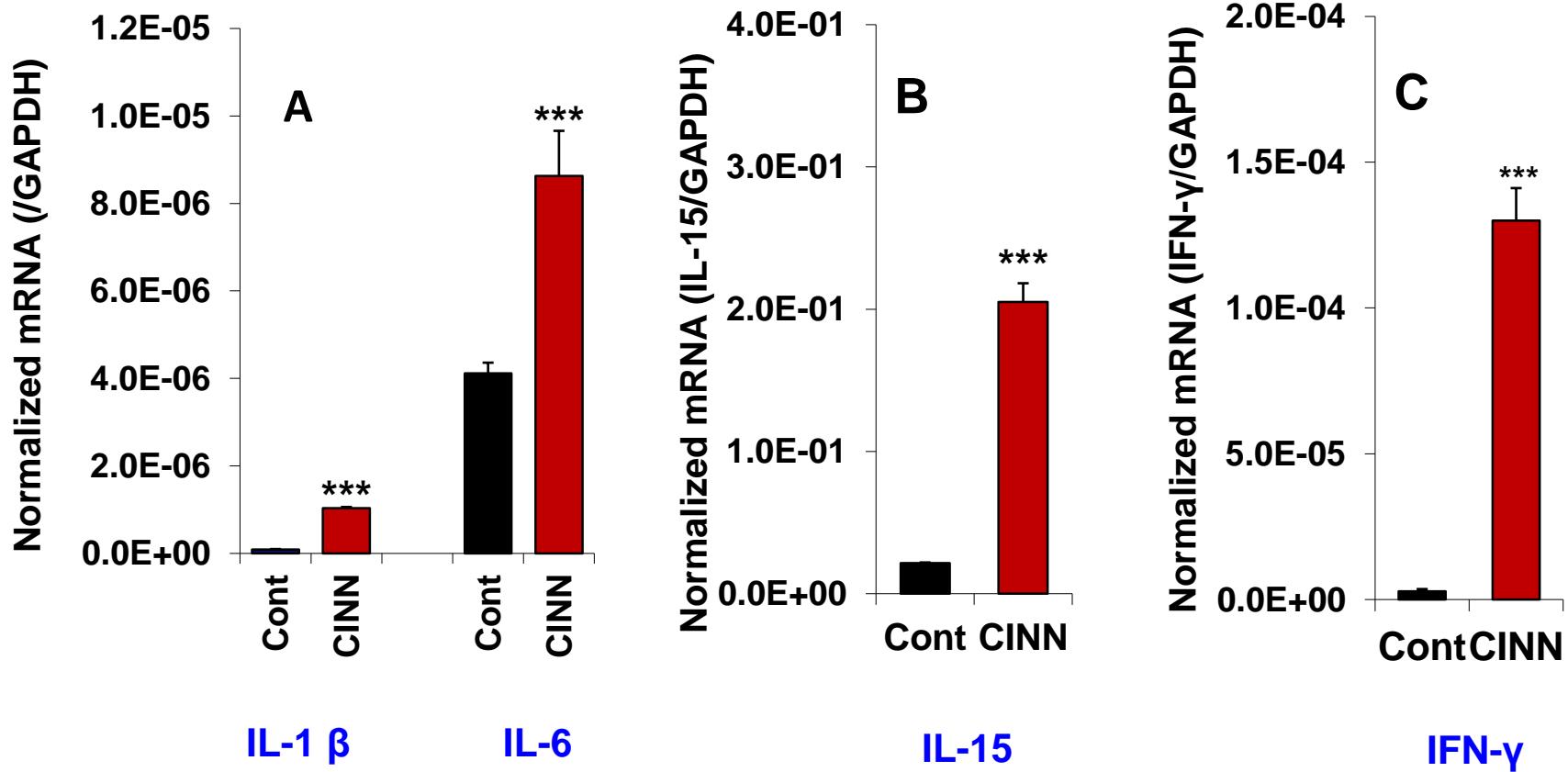


Fig 10. Effect of dietary CINN on intestinal cytokine transcript levels.

SUMMARY

In vitro:

CINN induced significantly higher splenocyte proliferation, nitric oxide production, inhibition of chicken tumor cell growth, and exerted direct killing effect against *Eimeria* sporozoites.

In vivo:

CINN-fed birds showed 10~30% increases in body weight gain and 24 ~43% decreases in fecal oocysts production compared to the untreated birds following challenge infection with EA, EM or ET. All chickens produced higher IgY antibody titers against coccidia.

The levels of intestinal lymphocyte cytokine transcripts of IL-1 β , IL-6, IL-15, or IFN- γ were 2-5 folds higher and several pathways of metabolic and cellular immune response were altered by >2.0 fold in CINN-fed chickens compared to the controls.

Conclusion

- This study provides the first immunological evidence that cinnamaldehyde enhances innate immunity of chickens and increases local protective immunity against avian coccidiosis.
- Therefore, dietary supplementation of young broilers with cinnamaldehyde could be an alternative way to improve gut health and to decrease the use of drugs in poultry production.

Published Papers

Lillehoj HS, Kim DK, Bravo DM, Lee SH. Effects of dietary plant-derived phytonutrients on the genome-wide profiles and coccidiosis resistance in the broiler chickens. BMC Proc. 2011 Jun 3;5 Suppl 4:S34.

Lee, S.H., Lillehoj, H.S., Jang, S.I., Lee, K.W., Bravo, D., Lillehoj, E.P., 2011, Effects of dietary supplementation with phytonutrients on vaccine-stimulated immunity against infection with *Eimeria tenella*. Vet Parasitol 181, 97-105.

Lee, S.H., Lillehoj, H.S., Jang, S.I., Lee, K.W., Park, M.S., Bravo, D., Lillehoj, E.P., 2011, Cinnamaldehyde enhances in vitro parameters of immunity and reduces in vivo infection against avian coccidiosis. Br J Nutr 106, 862-869.

Lee, S.H., Lillehoj, H.S., Hong, Y.H., Jang, S.I., Lillehoj, E.P., Ionescu, C., Mazuranok, L., Bravo, D., 2010, In vitro effects of plant and mushroom extracts on immunological function of chicken lymphocytes and macrophages. Br Poult Sci 51, 213-221.

Lee, S.H., Lillehoj, H.S., Jang, S.I., Ionescu, C., Bravo, D. Effect of Dietary *Curcuma*, *Capsicum*, and *Lentinus*, on Enhancing Local Immunity against *Eimeria acervulina* Infection. J. Poultry Sci 47, 89-95.

Kim, DK, Lillehoj, HS, Lee, SH, Lillehoj, EP, Bravo, d. Improved resistance to *Eimeria acervulina* infection in chickens due to dietary supplementation with garlic metabolites. Brit J Nutr., In press.

Presented at conferences

Lee SH, Lillehoj HS, Jang SI, Kim DK, Jeong MS, Lillehoj EP, Bravo D. Supplementation of phytonutrients improves immune system and increases resistance to necrotic enteritis in young broiler chickens. PSA annual meeting, Georgia, July 9-12, 2012.

Lee SH, Lillehoj HS, Jang SI, Kim DK, Jeong MS, Lillehoj EP, Bravo D. Dietary supplementation of young broiler chickens with Capsicum and turmeric oleoresin increases resistance to necrotic enteritis. ASAS, Phoenix Arizona, July 15-19, 2012.

Lee SH, Lillehoj HS, Jang SI, Lee KW, Park MS, Bravo D. The synergistic effects of a plant-derived nutritional mixture on recombinant antigen vaccination against avian coccidiosis. AVMA/AAAP 2001 Scientific Program, POULTRY Section. Convention Center, St. Louis, MO, July 16-19, 2011.

Lee SH, Lillehoj HS, Jang SI, Lee KW, Kim DK, Park MS, Bravo D. Anethol Enhances *In Vitro* Parameters of Immunity and Augments *In Vivo* Protection Against Avian Coccidiosis. AVMA/AAAP 2001 Scientific Program, POULTRY Section. Convention Center, St. Louis, MO, July 16-19, 2011.

Lee Sung Hyen, Lillehoj Hyun S, Jang Seung, Lee Kyung Woo, Park Myeong Seon, Bravo David. Dietary cinnamaldehyde enhances intestinal protective immunity against *Eimeria acervulina*, *E. maxima* and *E. tenella* in broiler chickens. Joint Meeting of the ADSA, AMSA, ASAS and PSA, Joint Annual Meeting Denver, CO, July 11-15, 2010.

Lee Sung Hyen, Lillehoj Hyen S, Hong Yeong Ho, Bravo David. In vitro effects of plant and mushroom extracts on immunological function of chicken lymphocytes, and macrophages. Joint Meeting of the ADSA, AMSA, ASAS and PSA Denver CO, July 11-15, 2010.



Thank you so
much!